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Comparisons of Genetic Variance and Physiological Responses in Two Populations of Western Mosquitofish, Gambusia affinis

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Comparisons of Genetic Variance and Physiological Responses in Two Populations of Western Mosquitofish, *Gambusia affinis*.

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Brett Egger

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ABSTRACT

The study was conducted to determine the effects of lowered genetic variance in the Western mosquitofish (*Gambusia affinis*) on the physiological parameters of critical thermal maximum and minimum pH. Mosquitofish were obtained from two small central Illinois ponds, Lost Pond and Shadow Pond, by seines and dip nets. Once caught, fish were returned to the laboratory and acclimated for a minimum of five days at a temperature of 27°C and a pH of 8.25. At the beginning of each experimental run, fish were subjected to the physiological experiments for determination of critical thermal maximum and minimum pH, and then measured for length and weight. At the completion of each trial, mosquitofish were frozen at -70°C for later electrophoretic analysis. The electrophoretic analysis at eighteen loci using two buffers revealed that Lost Pond mosquitofish had a significantly higher mean heterozygosity of 0.118 ± 0.046 ($F_{1,128} = 9.64$, $P = 0.002$) than Shadow Pond mosquitofish 0.065 ± 0.027. The Lost Pond mosquitofish population had a mean number of alleles per locus of 1.4 ± 0.1 and six polymorphic loci (*G3pdh, Mpi, Pgdh, Ldh-1, Ldh-2, Np*), while the Shadow Pond population had a mean number of alleles per locus of 1.6 ± 0.1 and three polymorphic loci (*Mpi, Ldh-1, Ldh-2*). The physiological experiments show that Shadow Pond mosquitofish had a significantly higher mean CTMax of 42.2 ± 0.16°C ($Z = 4.46$, $P < 0.0000$) than Lost Pond mosquitofish 40.7 ± 0.26°C, while Lost Pond mosquitofish exhibited a significantly lower mean minimum pH of 1.42 ± 0.025 ($Z = 3.57$, $P = 0.0002$) than Shadow Pond mosquitofish 1.82 ± 0.025. An analysis of covariance showed that structural size ($F = 0.048$, $P = 0.827$) and heterozygosity ($F = 0.500$, $P = 0.685$) did not significantly affect CTMax but weight ($F = 6.241$, $P = 0.018$) and population ($F = 5.30$, $P = 0.028$) had a significant effect. An ANCOVA on minimum pH showed no significant effect of

/eight (F = 1.237, $P = 0.274$) or heterozygosity (F = 0.749, $P = 0.530$), a marginal affect of tructural size (F = 3.385, $P = 0.075$) and a significant effect of population (F = 72.652, P < .0001). Thus, because CTMax and minimum pH for both populations of mosquitofish from the vo sites was not correlated with heterozygosity, we may be observing physiological or genetic daptations in response to local environmental conditions.

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INTRODUCTION

Evolution is the change in the genetic composition of a population over time. A major mechanism of evolution is natural selection based upon differential reproduction of phenotypes. The smallest scale of evolution, microevolution, occurs when genetic change occurs in a few generations. Although natural selection is the main factor responsible for evolutionary change, microevolution can also occur due to gene flow, mutation, nonrandom mating and genetic drift. Where population levels are low, genetic drift (the random change in gene frequencies due to sampling error in the production of a new generation) may be responsible for a significant proportion of evolutionary change (Allendorf and Phelps, 1980). One type of genetic drift that can occur in a small population is a genetic bottleneck. A bottleneck is the sudden and drastic reduction in a population's size, due to a major population level factor such as climatic change, increased predation, disease or a catastrophic event (Meffe, 1986). A genetic bottleneck can lead to a reduction of genic diversity (loss of alleles) (Nei *et al.*, 1975; Leberg, 1992), and an increase in the probability of inbreeding, which can result in a decrease in heterozygosity and an associated increase in homozygosity. A loss in heterozygosity or genetic variability in a population due to a genetic bottleneck may decrease a population's ability to adapt (Meffe, 1986).

Increases in homozygosity have been shown to lead to a reduction in fertility and clutch size in the domestic chicken, *Gallus domesticus* (Jull, 1933), and a complete loss of reproductive fitness by inbreeding depression in the third generation of Japanese quail, *Coturnix coturnix japonica* (Sittman, 1966). In fish, reductions in heterozygosity have been associated with a decrease in fertility in the Zebra fish, *Brachydanio rerio* (Mrakovcic and Haley, 1979). Heterozygotic rainbow trout (*Salmo gairdneri*) were shown to use significantly less oxygen during development

and were significantly larger than homozygotic individuals, which suggests a relationship between a loss of metabolic efficiency and homozygosity (Danzmann *et al.*, 1987). In addition, rainbow trout showed significant declines in growth rate, fry survival, feeding efficiency and increased fry crippling with decreasing heterozygosity (Kincaid, 1976). Similarly, the loss of heterozygosity has also been associated with body deformities and increased asymmetry in rainbow trout (Aulstad and Kittelsen, 1971), as well as hatchery raised cutthroat trout (*Salmo clarki lewisi*) (Leary *et al.*, 1985). Lastly, it has been shown that decreases in developmental rate can be associated with increases in homozygosity in the rainbow trout; in 26 out of 43 comparisons of developmental rate, homozygous rainbow trout were observed to develop slower than heterozygous rainbow trout (Danzmann *et al.*, 1985).

An excellent situation for looking at the effects of genetic bottlenecks on reductions in heterozygosity may be found in small, isolated, man made ponds in central Illinois. Two such ponds are Lost Pond and Shadow Pond. Lost Pond is a small, 6100 m^2, pond with a maximum depth of 2.58 m located near Lake Shelbyville in Shelby County, Illinois. It is located adjacent to several agricultural fields from which it receives agricultural runoff. Lost Pond is located at the northernmost part of the natural range of mosquitofish in Illinois (Smith, 1979), however, they have been introduced into areas as far north as southern Michigan (Krumholz, 1948). Lost Pond is situated at the crest of a north-facing slope and has no physical contact with Lake Shelbyville, the closest body of water, even during periods of high flooding, thus preventing any gene flow between mosquitofish populations of Lake Shelbyville and Lost Pond. Characteristics of a fish population that may have undergone a genetic bottleneck were observed in the mosquitofish population in Lost Pond. The mosquitofish population has drastic population fluctuations, in a

year's sampling time as many as 150 and as few as 0 individuals were caught at various sampling times. (Egger, personal observations). In addition, fish collected from Lost Pond also produced offspring with spinal deformities when bred in the laboratory, which may indicate developmental instability (Egger, personal observations).

Fish from Lost Pond were compared to a more southern population of mosquitofish found in Shadow Pond near Lake Paradise in Coles County, Illinois. Shadow Pond is a larger pond with an area of approximately 12,000 m^2 and a maximum depth of about one meter. It is found in a natural park setting and is situated 50 m from Lake Paradise. During periods of high flooding, Shadow Pond is connected to Lake Paradise, allowing for gene flow between mosquitofish populations of the two bodies of water. Although anecdotal, evidence that gene flow might be occurring between the two areas exists because drastic population fluctuations and deformities were not observed in the population of mosquitofish from Shadow Pond. Thus, gene flow appears to be occurring during periods of flooding which could be reducing the chances of the Shadow Pond population of mosquitofish from experiencing a genetic bottleneck.

Since a decrease in heterozygosity has been shown to directly affect fitness and development, the focus of my research was to determine if increases in homozygosity within a bottlenecked mosquitofish (*Gambusia affinis*) population (Lost Pond) will have an effect on the fish's genetic variability and physiological response. Specifically the study will address the following working questions:

1) Is there a difference in mean heterozygosity, number of polymorphic alleles and mean number of alleles per locus between the two mosquitofish populations from Lost Pond and Shadow Pond?

2) Is there a physiological difference in the ability of the two mosquitofish populations to respond to the physiological parameters of critical thermal maximum, and minimum pH?

3) Is there a correlation between the observed genetic compositions of the mosquitofish populations and there ability to respond to the physiological parameters of critical thermal maximum and minimum pH?

Critical thermal maximum and minimum pH were the physiological parameters chosen because large temperature and pH fluctuations have been observed in small ponds (Cole, 1979; Maberly, 1996) and differences in physiological response caused by genetic variability between fish populations are likely to be observed at physiological and environmental extremes.

Organism:

Western mosquitofish (*Gambusia affinis)* are small livebearing fish in the family Poeciliidae, in which development of the embryos takes place inside the female, producing freeswimming fry (Constantz, 1989). The fish can reach sexual maturity in 4-6 weeks and bear fry in 21-28 days after fertilization (Krumholz, 1948). Mosquitofish exhibit sexual dimorphism with males being smaller and having a specialized anal fin called a gonopodium which they use in sperm transfer (Krumholz, 1948; Constantz, 1989). Male maturity can be determined when the gonopodium is clear and spike like with a small spine visible at the tip (Meffe, 1992). Females are larger than males and lack a gonopodium. Upon maturation, females produce a dark spot by the anal fin and yolked eggs are visible in the abdomen (Meffe, 1992).

Mosquitofish (*G. affinis and G. holbrooki*) are found historically from southern New Jersey to Mexico and throughout the Mississippi River basin to southern Illinois and Indiana (Meffe, 1991).

The range of mosquitofish has increased due to its use as a mosquito control agent, and mosquitofish can now be found in temperate regions throughout the world (Krumholz, 1948). Poeciliids are generally tolerant of extreme values of temperature, oxygen levels, and other abiotic factors. Because of their small size, viviparous reproductive habits, and short generation times, mosquitofish are able to reproduce quickly in a variety of habitats (Moyle and Cech, 1988), which makes them a suitable organism for studies on life history (Stearns, 1983; Meffe, 1991; Scribner *et al.*, 1992; Scribner, 1993; Mulvey *et al.*, 1994; Weeks and Meffe, 1996), population genetics (Smith *et al.*, 1983; Feder *et al.*, 1984; Robbins *et al.*, 1987; Smith *et al.*, 1989; Hernandez-Marich and Smith, 1990), and ecotoxicology (Newman et al., 1989; Diamond *et al.*, 1991; Lee *et al.*, 1992; Newman and Aplin, 1992; Heagler *et al.*, 1993; Keklak *et al.*, 1994; Mulvey *et al.*, 1995).

There have been numerous studies using mosquitofish that have looked at the association between environmental stressors and genetics. One such study looked at the effects of long term exposure of mosquitofish to uranium (Keklak *et al.*, 1994). Mosquitofish collected from a site contaminated by uranium exhibited lower genetic variation than mosquitofish that were from an uncontaminated site. A stress test exposing F2 generation fish to 2.57 mg/L of uranium exhibited a 96% to 98% mortality in the F2 fish from the uncontaminated site and only a 25% to 57% mortality in the F2 fish from the uranium contaminated site. The increased tolerance in mosquitofish from the contaminated site was thought to have been due to a genetically based selection associated with the toxicant (Keklak *et al.*, 1994).

Studies that have investigated long term effects of inorganic mercury on mosquitofish populations have observed histological changes in the lamellae (Paulose, 1987) and in the energy

producing metabolic pathways (Kramer *et al.*, 1992). Specifically, fusion, hyperplasia and desquamation occurred in the lamellae (Paulose, 1987), while decreases in the concentrations of glucose-6-phosphate (G6P) and fructose-6-phosphate (F6P) and increases in the concentrations of pyruvate, α-ketoglutarate, succinate and malate were observed in the metabolic pathways (Kramer *et al.*, 1992). In addition, the Krebs cycle activity increased due to increased energy needs associated with homeostasis in a stressful environment. Thus, long term exposure to mercury reduced glycolytic activity and caused a response similar to that elicited by starvation (Kramer *et al.*, 1992)

Experiments involving acute exposure to toxicants using mercury, arsenate and salinity have been performed investigating the correlation between time-to-death (TTD) and specific enzyme loci. Allozyme genotypes at three loci: glucosephosphate isomerase-2 (*GPI-2*), isocitrate dehydrogenase-1 (*ICD-1*) and malate dehydrogenase-1 (*MDH-1*) were used to determine if there was a significant correlation between TTD of mosquitofish and acute toxicant exposure (Diamond *et al.*, 1989). The shortest median TTD was strongly associated with the homozygous genotypes ($GPI\text{-}2^{38}/GPI\text{-}2^{38}$) for mercury (Diamond *et al.*, 1989; Heagler *et al.*, 1993; Mulvey *et al.*, 1995) and arsenate (Newman *et al.*, 1989); and ($GPI\text{-}B^{66}/GPI\text{-}B^{66}$) for salinity (Kandl and Thompson, 1996). Thus, the homozygous genotypes, $GPI\text{-}2^{38}$ and $GPI\text{-}B^{66}$, are strongly associated with low stress tolerance and have been found in significantly lower frequencies from contaminated sites (Heagler *et al.*, 1993).

Population genetics experiments using temperature as a stressor have been performed on mosquitofish from laboratory and field settings. Mosquitofish from cooler environments had an expected lower critical thermal maximum than mosquitofish from warmer environments (Meffe *et*

al., 1995). Studies that have examined the heterozygosity of mosquitofish from differing thermal environments have found that overall heterozygosity was higher in populations from warmer environments compared to cooler environments (Feder *et al.*, 1984; Meffe *et al.*, 1995) and that juveniles tended to be less heterozygous than adults (Feder *et al.*, 1984). When mosquitofish were reared at temperatures of 25°C and 32°C until 10 weeks of age, heterozygous individuals had faster growth rates than homozygous fish at the higher temperature, but no difference was observed in growth rates at the lower temperature (Mulvey *et al.*, 1994). Thus, the results using stressors such as temperature, inorganic mercury, arsenate and salinity stress seem to indicate that a higher level of multilocus heterozygosity would benefit organisms living in stressful situations and possibly confer increased fitness in the form of higher survival rates (Allendorf and Leary, 1986; Meffe *et al.*, 1995).

METHODS & MATERIALS

One hundred and fifty individual *Gambusia affinis* were obtained from Lost Pond during December 1994, and seventy-five were captured from Shadow Pond on May 28, 1996 using seines and dip nets. Upon capture, the fish were brought to the Fish Ecology Laboratory at Eastern Illinois University at which time they were placed in 10 gallon aquaria. Mosquitofish from Lost Pond were kept in the laboratory for one year and acclimated at their preferred temperature of 27°C (Coutant, 1977), and a pH of 8.25. Shadow Pond mosquitofish were acclimated for a minimum of 5 days at the same temperature and pH as the mosquitofish from Lost Pond. Water temperature and pH were checked daily with a mercury thermometer and pH meter respectively. Fish were fed *ad libitum* twice per day and kept on a 24-hour light cycle. Fish were not fed for two days prior to testing to insure that all fish had emptied their intestinal tract.

The physiology experiments began by placing individual fish in a 500 ml beaker containing 300 ml of water. A glass tube was affixed to the side of the beaker and an air hose inserted into the tube, to provide aeration and circulation of the water. Critical thermal maximum was determined by raising the water temperature 0.5°C per minute from 27°C to experimental endpoint (Paladino *et al.*, 1980; Peterson, 1993). The temperature increase of 0.5°C per minute allowed body temperature to follow test temperature without a lag period (Paladino *et al.*, 1980). The experimental endpoint or critical thermal maximum is defined as the point when the fish shows any of the following responses: 1) rapid shuddering motions, 2) agape mouth, 3) loss of righting response or 4) a complete loss of equilibrium (Fry *et al.*, 1946; Paladino *et al.*, 1980). Twenty and twenty-one mosquitofish were tested for critical thermal maximum from Shadow Pond and

Lost Pond respectively.

The experiment to determine minimum pH utilized the same apparatus as described above, however the temperature remained at a constant 27° C throughout the experiment. In the minimum pH experiments, acidity of the water was increased from the acclimation pH of 8.25 at a rate of 0.025-0.030 pH units per minute with H_2SO_4 until an experimental endpoint like that used for critical thermal maximum was observed (Peterson *et al.*, 1989; Falter and Cech, 1993). Seventeen and twenty-one mosquitofish were tested for minimum pH from Shadow Pond and Lost Pond respectively.

Upon termination of each experimental run, mosquitofish were individually weighed to the nearest tenth of a gram and total body length was measured to the nearest millimeter. Mosquitofish were placed in individually marked plastic microfuge tubes and frozen at -70°C in the laboratory until processed for electrophoretic analysis. Whole fish were ground in the tubes using 0.50 ml of a 0.01 M tris-EDTA buffer (pH 7.5) (McClenaghan *et al.*, 1985). Filter paper wicks were placed into the tubes to absorb extracts. The wicks were then inserted into 12% starch gels with either continuous tris citrate or discontinuous tris citrate buffer (McClenaghan *et al.*, 1985). Buffers and corresponding protein systems included: continuous tris citrate (pH 8.0) (Selander *et al.*, 1971) for α-glycerol-3-phosphate dehydrogenase (*G3PDH*; EC 1.1.1.8), creatine kinase (*CK-A, B*; EC 2.7.3.2), glucosephosphate isomerase (*GPI-B*; EC 5.3.1.9), isocitrate dehydrogenase (*s-IDHP*; EC 1.1.1.42), mannose-6-phosphate isomerase (*MPI*; EC 5.3.1.8), 6-phosphogluconate dehydrogenase (*PGDH;* EC 1.1.1.44), and phosphoglucomutase (*PGM*; EC 2.7.5.1); and discontinuous tris citrate (buffer C-) (Ayala *et al.*, 1972) for fumarate hydratase (*FH*; EC 4.2.1.2), lactate dehydrogenase (*LDH-A, B*; EC 1.1.1.27), malate dehydrogenase (*s-*

MDH-A, B; EC 1.1.1.37), malic enzyme (*s-MEP*; EC 1.1.1.40), and nucleoside phosphorylase (*NP*; EC 2.4.2.1). Gels were run using an electric current of 45 amps for continuous tris citrate and 40 amps for discontinuous tris citrate for 16 hours then sliced and stained for the proteins (McClenaghan *et al.*, 1985).

A Mann-Whitney U test was used to determine if significant differences for both critical thermal maximum and minimum pH existed between mosquitofish populations (NWA Statpak, 1985). An analysis of variance was used to determine significant differences in heterozygosity between populations (Systat., 1996). Spatial heterogeneity in allelic frequencies was determined by contingency chi-square analysis. Genetic population structure was quantified by Wright's (1965, 1969) *F*-statistics as modified by Nei (1977), and Modified Rogers genetic distance (Wright, 1978). *F*-statistics, a Modified Rogers genetic distance and the contingency chi-square analysis were all performed using BIOSYS-1 (Swofford and Selander, 1981). An analysis of covariance was used to determine if structural size, weight or heterozygosity had an effect on critical thermal maximum or minimum pH (Systat., 1996). The ANCOVA used four independent variables in each model, heterozygosity class and population were categorical variables and structural size and weight were the covariates (Systat., 1996). Structural size was estimated as the residuals from a regression of length as a function of weight. Thus, structural size and weight are not correlated measures. Statistical significance was assumed for all tests if $P < 0.05$, and all means were expressed with one standard error.

RESULTS

Lost Pond mosquitofish had a mean heterozygosity of 0.118 $\pm$ 0.046, whereas Shadow Pond mosquitofish showed a mean heterozygosity of 0.065 $\pm$ 0.027. An ANOVA showed a statistically significant difference between mosquitofish populations ($F_{1,128} = 9.64$, $P = 0.002$) in mean multilocus heterozygosity.

The allelic frequencies of polymorphic loci for Lost Pond and Shadow Pond are shown in table 1. Of the 18 loci investigated 6 loci were polymorphic at the 0.95 level in the Lost Pond population (*G3pdh, Mpi, Pgdh, Ldh-1, Ldh-2, Np*) and 3 loci were polymorphic in the Shadow Pond population (*Mpi, Ldh-1, Ldh-2*). An ANOVA showed no significant difference in mean number of alleles per locus between the mosquitofish population from Lost Pond (1.4 $\pm$ 0.1) and the Shadow Pond mosquitofish population (1.6 $\pm$ 0.1). The Lost Pond population was in Hardy-Weinberg equilibrium for all loci (Table 2). Similarly, all loci from the Shadow Pond population were in Hardy-Weinberg equilibrium except PGDH ($\chi_1^2 = 17.987$, $P < 0.0001$) (Table 3). The Modified Rogers genetic distance between the populations was 0.185. Of the F_{is} values for the mosquitofish population from Lost Pond, *Pgdh* was statistically significant, while *Ldh-1* and the mean F_{is} over all loci were marginally significant (Table 4). Within Lost Pond, *G3pdh, Ldh-1* and *Ldh-2* showed marginally significant F_{is} values (F_{is} = -0.278, 0.264 and -0.271 respectively; χ_1^2 = 3.55, 3.69 and 3.82 respectively; P = 0.0594, 0.0546 and 0.0504 respectively). The mean F_{is} over all loci was not significant in Lost Pond. Within Shadow Pond, *PGDH* had a significant F_{is} value ($F_{is} = 0.486$, $\chi_1^2 = 17.95$, $P < 0.0001$) and the mean F_{is} over all loci for Shadow Pond was found to be significant ($F_{is} = 0.072$, $\chi_{11}^2 = 21.71$, $P = 0.0267$). F_{ST} values of *G3pdh, Ldh-1, Ldh-2* were all highly significant ($P < 0.0001$) while *Pgdh* was marginally significant ($P = 0.050$). A

significant proportion of the total genetic variance was held between populations (F_{ST} = 0.162. P < 0.0001).

The mosquitofish from Lost Pond had a mean critical thermal maximum of 40.7 ± 0.26°C, while individuals from the Shadow Pond population exhibited a mean critical thermal maximum of 42.2 ± 0.16°C (fig. 1). A one-sided Mann-Whitney U test showed a statistically significant difference (Z = 4.46, $P < 0.0000$) between the two populations in critical thermal maximum. The mean minimum pH of the mosquitofish from Lost Pond was 1.42 ± 0.025, and the mean minimum pH of the population of mosquitofish at Shadow Pond was 1.82 ± 0.025 (fig. 2). A one-sided Mann-Whitney U nonparametric test showed a statistically significant difference (Z = 3.57, P = 0.0002) between the two populations in regard to minimum pH.

An ANCOVA showed that structural size (F = 0.048, $P = 0.827$) and heterozygosity (F = 0.500. $P = 0.685$) did not significantly affect CTMax, but weight (F = 6.241, $P = 0.018$) and population (F = 5.30, $P = 0.028$) had a significant effect. An ANCOVA on only females from both populations showed that only population (F = 7.78, $P = 0.01$) had a significant effect on CTMax whereas heterozygosity (F = 1.15, $P = 0.35$), structural size (F = 0.01, $P = 0.94$) and weight (F = 2.08, $P = 0.17$) had no significant effect on CTMax. An ANCOVA on minimum pH showed no significant effect of weight (F = 1.237, $P = 0.274$) or heterozygosity (F = 0.749, P = 0.530), a marginal effect of structural size (F = 3.385, $P = 0.075$) and a significant effect of population (F = 72.652, $P < 0.0001$).

DISCUSSION

I hypothesized that the population of mosquitofish from Lost Pond had undergone a genetic bottleneck. If a genetic bottleneck had occurred in the Lost Pond population of mosquitofish, one would expect to see reductions in mean heterozygosity, percent polymorphic loci and mean number of alleles per locus (Nei *et al.*, 1975). Lost Pond mosquitofish had more polymorphic loci and had a statistically higher mean heterozygosity than the Shadow Pond mosquitofish. Thus, there is no evidence to support the hypothesis that the population of mosquitofish from Lost Pond underwent a genetic bottleneck.

The high level of heterozygosity in the Lost Pond mosquitofish population may be due to the fact that the founding population of mosquitofish in Lost Pond may have had a larger amount of heterozygosity compared to the founding population in Shadow Pond. The mean heterozygosity estimates observed in both Lost Pond and Shadow Pond fall within the range of mean heterozygosities values observed for various mosquitofish populations (0.055-0.124) and are lower than the estimate determined for a founding mosquitofish population from Texas (0.146; Smith *et al.*, 1989). Even if a genetic bottleneck had occurred in either population there are reasons why there would be no observed reductions in mean heterozygosity. Heterozygosity can increase in a population after a brief bottleneck due to the increased frequency of formerly rare alleles (Wright, 1931) or by the conversion of epistatic genetic variance to additive genetic variance (Cheverud and Routman, 1996). Also, female mosquitofish can be multiply inseminated and store sperm from 1-8 months (Krumholz, 1948; Greene and Brown, 1991), this may allow female mosquitofish to carrying sperm from multiple males through a bottleneck. So even drastic reductions in a mosquitofish population may not have a significant reduction in heterozygosity or

effective population size (Chesser *et al.*, 1984; Robbins *et al.*, 1987).

Mosquitofish from Shadow Pond had a statistically higher CTMax than mosquitofish from Lost Pond, which could be a result of differing environmental conditions in the ponds. Mosquitofish from Lost Pond and Shadow Pond had mean CTMax (40.7°C and 42.4°C respectively) (fig. 1) similar to mean CTMax of mosquitofish from South Carolina (between 40-41°C) (Meffe *et al.*, 1995). Mosquitofish generally have a higher CTMax than other fish species found in similar habitats or latitudes (Becker and Genoway, 1979; Peterson, 1993; Heath et al., 1994). One reason for a higher mean critical thermal maximum in mosquitofish could be due to their behavior as surface swimmers as well as their ability to inhabit the shallow areas near shore (Krumholtz, 1948). These characteristics would subject them to higher temperatures, which could make them more tolerant of higher (stressful) temperatures. Shadow Pond has a maximum depth of about one meter (Mike Mounce per. comm.), with a larger percentage of the pond being very shallow. This would result in higher temperatures and greater temperature fluctuations than would be observed in Lost Pond, which has a maximum depth of 2.58 meters and few shallow areas. Thus, mosquitofish living within Shadow Pond may have an increased tolerance to extreme temperatures due to a physiological adaptation for their specific environment.

Most studies dealing with acute pH stress have used maximum pH rather than minimum pH (Falter and Cech, 1991). In this study, minimum pH was used rather than maximum pH because of the potential for acid rain effects to cause damage to both the environment and the organisms living within the environment. Lost Pond had a pH of 9.20 and Shadow Pond had a pH of 8.65, when measured in May 1996. However the mosquitofish at Lost Pond had a significantly lower mean pH than the Shadow Pond mosquitofish population. The significantly lower minimum pH

of mosquitofish at Lost Pond may be due to its location. Lost Pond is in close proximity to agricultural fields that could subject it to seasonal agricultural runoff which could decrease the pH of the aquatic environment by as much as three pH units during certain times of the year (Kopacek *et al.*, 1995; Maberly, 1996). Thus, mosquitofish from Lost Pond may have either a physiological or genetic adaptation for their local conditions.

When an analysis of covariance was used to determine if structural size, weight or heterozygosity had an effect on critical thermal maximum, only weight and population were significant. Weight of an individual has a positive correlation to CTMax (independent of population) with the same relationship observed in both populations. Shadow Pond mosquitofish had a significantly higher CTMax than Lost Pond fish which could be due to the fact that only female mosquitofish, which have on average a larger body weight, were used in CTMax tests from Shadow Pond whereas only half of the mosquitofish used for CTMax determination from Lost Pond were females. A second ANCOVA using just females from both populations showed that weight did not affect a female's CTMax but significant differences were observed between populations. Thus, the differences in temperature tolerances of female mosquitofish observed between the ponds may be attributed to adaptation to local conditions. For minimum pH neither weight nor heterozygosity had a significant effect in an analysis of covariance. Structural size was marginal and significance may have been obtained if a larger sample size had been used, suggesting that structural size may have had an effect on a minimum pH tolerance. The differences in the tolerances of mosquitofish to low minimum pH between the ponds may be attributed to local adaptation. Lost Pond may experience drastic changes in pH because it is a small pond that is closely associated to several agricultural fields from which it receives runoff.

These fluctuations in pH could cause populations of mosquitofish in Lost Pond to be more tolerant of pH stress over time. This tolerance of pH stress by mosquitofish from Lost Pond may have a genetic component associated with fluctuating pH in Lost Pond.

Although heterozygosity has been strongly associated with fitness components such as fertility, clutch size and growth rates (Kincaid, 1976; Mrakovic and Haley, 1979) and theoretically associated with a population's ability to adapt (Meffe, 1986), this study found no association between electrophoretic heterozygosity and adaptiveness tested by CTMax and minimum pH. The reason for this may be due to the small number of loci examined when compared to the genome (Powell, 1983; Leberg, 1992). More likely, the reason that CTMax and minimum pH are not correlated with heterozygosity in these mosquitofish populations may be due to the fact that we are observing physiological adaptations, which may have a genetic component, caused by the mosquitofish populations adapting to local conditions. There have been other studies that have shown no positive correlation between electrophoretic heterozygosity and fitness components (McAndrew et al., 1982; Booth et al., 1990; Whitlock, 1993), as well as physiological stressors such as uranium (Keklak *et al.*, 1994). A second generation test on CTMax and minimum pH may give an indication if there is a genetic component to these stressors. If second generation mosquitofish from Lost Pond and Shadow Pond exhibit a mean minimum pH and CTMax similar to the mean minimum pH and CTMax of the parental generation, then it can be assumed that local adaptation in Lost Pond and Shadow Pond has selected genes that favor either a low pH tolerance for Lost Pond mosquitofish or higher temperature tolerance for Shadow Pond mosquitofish. Further studies on the loss of heterozygosity and its effect on physiological stressors need to be done before a complete understanding of how the loss of heterozygosity directly affects a

population's ability to adapt.

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Table 1: Allelic Frequencies in populations of mosquitofish from Lost Pond and Shadow Pond

Locus			Lost Pond	Shadow Pond
Pgm-1	N		51	77
		A	0.01	0
		B	0.99	1
Pgm-3	N		49	70
		A	0	0.021
		B	1	0.979
G3pdh	N		46	60
		A	0.391	0.042
		B	0.609	0.958
Mpi	N		49	74
		A	0.214	0.176
		B	0.786	0.824
Idhp	N		51	72
		B	1	0.993
		C	0	0.007
Pgdh	N		50	76
		A	0.08	0.026
		B	0.92	0.974
Mdh-1	N		53	76
		A	0	0.013
		B	1	0.987
Mdh-2	N		53	77
		B	1	0.994
		C	0	0.006
Ldh-1	N		53	77
		B	0.849	0.532
		C	0.151	0.468
Ldh-2	N		52	77
		B	0.269	0.883
		C	0.731	0.117
Np	N		10	32
		B	0.85	0.953
		C	0.15	0.047

Gpi-1, *Gpi-2*, *Ck-1*, *Ck-2*, *Pgm-2*, *Fh* and *Mep* were monomorphic in both populations.

Table 2: Deviation from Castle-Hardy-Weinberg equilibrium for polymorphic loci in Lost Pond.

Locus	Genotype	Observed	Expected	χ^2	DF	P
Pgm-1	A-A	0	0.005			
	A-B	1	0.990			
	B-B	50	50.005			
				0.005	1	0.944
G3pdh	A-A	4	7.043			
	A-B	28	21.913			
	B-B	14	17.043			
				3.549	1	0.060
Mpi	A-A	2	2.250			
	A-B	17	16.500			
	B-B	30	30.250			
				0.045	1	0.832
Pgdh	A-A	0	0.320			
	A-B	8	7.360			
	B-B	42	42.320			
				0.378	1	0.539
Ldh-1	B-B	40	38.208			
	B-C	10	13.585			
	C-C	3	1.208			
				3.691	1	0.055
Ldh-2	B-B	1	3.769			
	B-C	26	20.462			
	C-C	25	27.769			
				3.810	1	0.051
Np	B-B	7	7.225			
	B-C	3	2.550			
	C-C	0	0.225			
				0.311	1	0.577

Table 3: Deviation from Castle-Hardy-Weinberg equilibrium for polymorphic loci in Shadow Pond.

Locus	Genotype	Observed	Expected	χ^2	DF	P
Pgm-3	AA	0	0.032			
	AB	3	2.936			
	BB	67	67.032			
				0.034	1	0.855
G3pdh	AA	0	0.104			
	AB	5	4.792			
	BB	55	55.104			
				0.113	1	0.736
Mpi	AA	3	2.284			
	AB	20	21.432			
	BB	51	50.284			
				0.331	1	0.565
Idhp	B-B	71	71.003			
	B-C	1	0.993			
	C-C	0	0.003			
				0.004	1	0.953
Pgdh	A-A	1	0.053			
	A-B	2	3.895			
	B-B	73	72.053			
				17.987	1	0.000
Mdh-1	A-A	0	0.013			
	A-B	2	1.974			
	B-B	75	75.013			
				0.013	1	0.908
Mdh-2	B-B	76	76.003			
	B-C	1	0.994			
	C-C	0	0.003			
				0.003	1	0.954
Ldh-1	B-B	25	21.831			
	B-C	32	38.338			
	C-C	20	16.831			
				2.104	1	0.147
Ldh-2	B-B	61	60.052			
	B-C	14	15.896			
	C-C	2	1.052			
				1.096	1	0.295
Np	B-B	29	29.070			
	B-C	3	2.859			
	C-C	0	0.070			
				0.077	1	0.781

Table 4: Summary of F-statistics and total limiting variance at all loci for mosquitofish populations at Lost Pond and Shadow Pond.

Locus	F_{IS}	F_{IT}	F_{ST}	σ_T^2
Pgm-1	-0.010	-0.005	0.005	0.010
Pgm-3	-0.022	-0.011	0.011	0.021
G3pdh	-0.244	-0.020	0.180***	0.339
Mpi	0.015	0.017	0.002	0.314
Idhp	-0.007	-0.003	0.003	0.007
Pgdh	0.061**	0.075	0.014	0.101
Mdh-1	-0.013	-0.007	0.007	0.013
Mdh-2	-0.007	-0.003	0.003	0.006
Ldh-1	0.199*	0.293	0.117***	0.427
Ldh-2	-0.136	0.302	0.386***	0.488
Np	-0.143	-0.109	0.030	0.178
Mean	-0.031*	0.136	0.162***	0.173

* $0.1 > P > 0.05$, **$P < 0.0005$, ***$P < 0.0001$

The significance of F_{IS} determined by χ^2 approximation (Nei and Chesser, 1983).

The significance of F_{ST} determined by χ^2 contingency analysis (Workman and Niswander, 1970).

Figure 1: Mean critical thermal maxima (± 2 SE) of mosquitofish populations from Lost Pond and Shadow Pond from central Illinois.

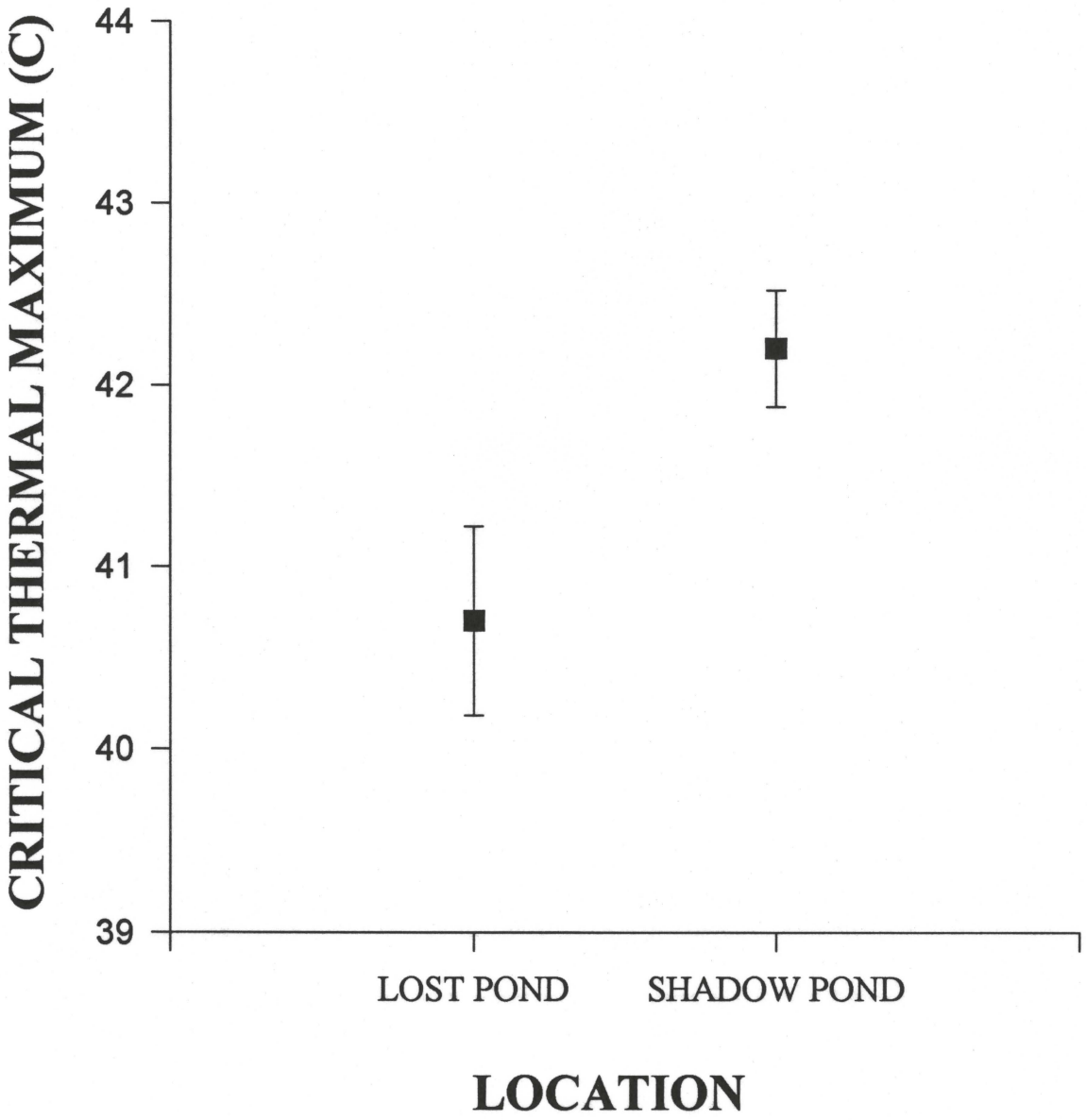
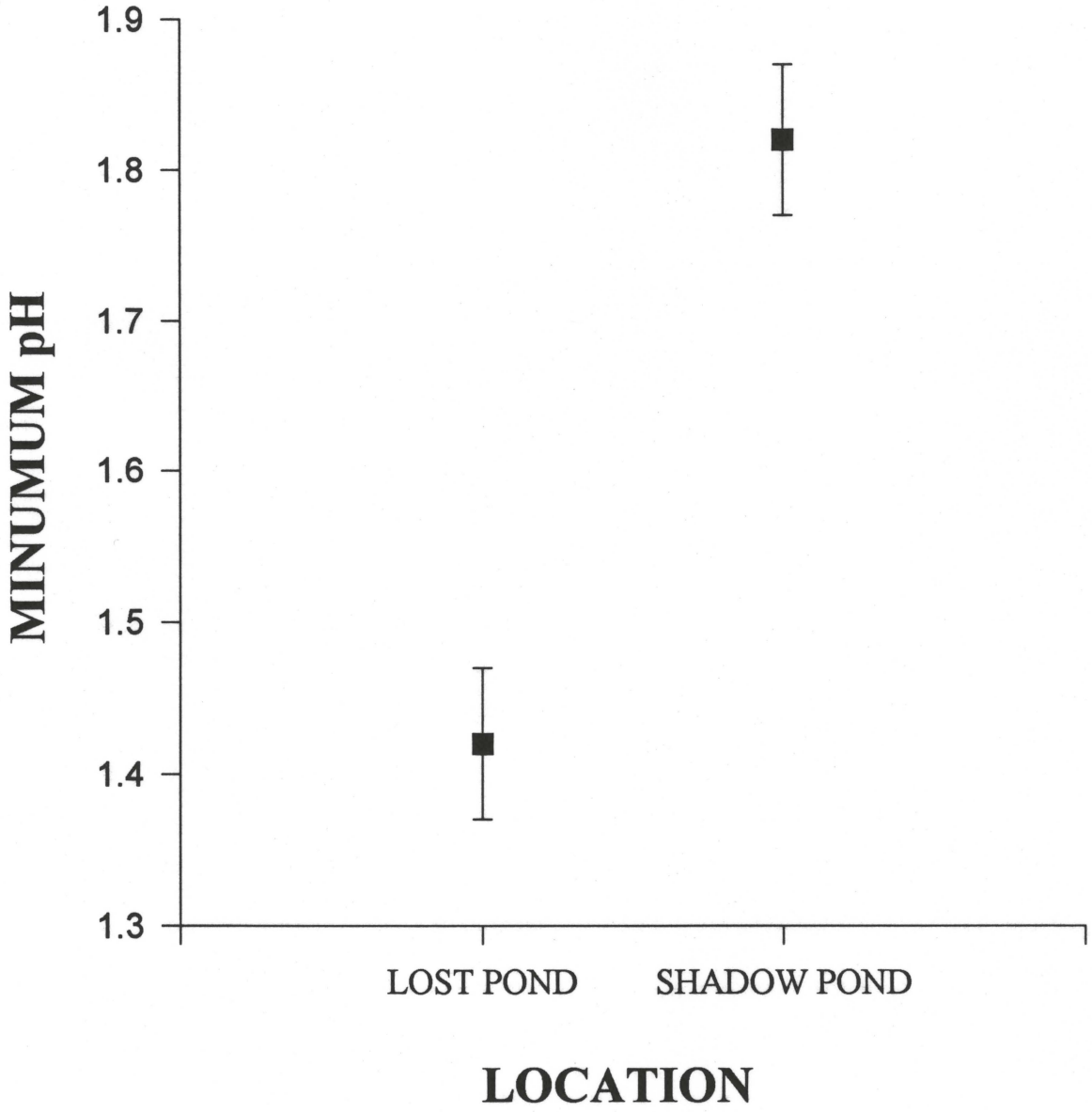

CRITICAL THERMAL MAXIMUM (C)
44
43
42
41
40
39
LOST POND
SHADOW POND
LOCATION

Figure 2: Mean minimum pH (± 2 SE) for mosquitofish populations from Lost Pond and Shadow Pond from central Illinois


1.9
1.8
1.7
1.6
1.5
1.4
1.3
MINUMUM pH
LOST POND
SHADOW POND
LOCATION